The Egyptian Journal of Medical Human Genetics (2011) **12**, 151–156

Ain Shams University

The Egyptian Journal of Medical Human Genetics

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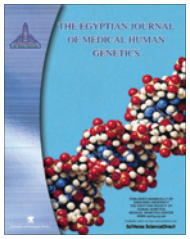

ORIGINAL ARTICLE

Genetic association between common beta-2 adrenoreceptor polymorphism and asthma severity in school-age children

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Received 27 September 2010; accepted 21 December 2010
Available online 27 August 2011

KEYWORDS

Asthma;
Beta-2 adrenoceptor gene polymorphisms;
Arginine 16/Glycine

Abstract Recent studies have suggested that polymorphism of the beta-2 adrenergic receptor (β2AR) gene at codon 16 affect an individual's airway responsiveness. This study aimed to evaluate the association between beta-2 adrenoreceptor genotypes at position 16 and asthma severity among 59 school-aged children. They were divided into two groups: control group including 19 healthy children and 40 asthmatic children as the study group. The study group was also divided into 20 mild asthmatic children and the remaining 20 patients suffered from severe asthma. Blood samples were collected from Chest Department, Pediatrics Hospital, Ain Shams University, from February 2008 to March 2009. Molecular analysis was performed at Science Faculty, Ain Shams University, Cairo, Egypt. Venous blood sample were collected and genotyping of β2AR gene polymorphism at position 16 was identified by polymerase chain reaction–restriction fragment length polymorphism analysis, using the *Nco*I restriction enzyme. We found a highly statistically significant difference of polymorphisms' distribution of β2AR gene at codon 16 among asthmatic patients and control subjects ($\chi^2 = 11.904$; $P = 0.0026$), also among severe asthmatics and mild/moderate asthmatics

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Peer review under responsibility of Ain Shams University.
doi:10.1016/j.ejmhg.2011.07.003

($\chi^2 = 10.108$; $P = 0.0064$). There was a strong association of heterozygous Arg16Gly of β2AR with severe asthmatics rather than that in control subjects (70% vs. 5.3%, $P < 0.001$), with odd ratio (OR) 42 (95% confidence interval (CI): 4.520–390.297), the highest OR in heterozygous Arg16Gly (42) suggests a dominant mode of action of the heterozygous Arg16Gly in development of asthma severity. So, we concluded that heterozygous Arg16Gly of β2AR gene appeared to be an important genetic factor in the expression of asthma severity.

1. Introduction

Asthma is a complex genetic disorder that is characterized by airway inflammation and reversible airflow obstruction [1]. Asthma prevalence has increased very considerably in recent decades such that it is now one of the commonest chronic disorders in the world. Asthma prevalence increased significantly, especially in developed countries, and particularly in children. What caused this increase is currently unknown [2]. Approximately 300 million people worldwide currently have asthma, and its prevalence increases by 50% every decade. In North America, 10% of the population has asthma. The increasing number of hospital admissions for asthma, which are most pronounced in young children, reflect an increase in severe asthma and poor disease management. Worldwide, approximately 180,000 deaths annually are attributable to asthma [3–5].

Recent study was performed in Cairo, Egypt to ascertain the prevalence of asthma among children. It revealed that the overall prevalence of wheezing in the last year was 14.7% and of physician-diagnosed asthma was 9.4% [6].

Beta-2 adrenergic receptors are iniquitous throughout the human body and are classified into three distinct subtypes (β1, β2, and β3) on the basis of their function, agonist-binding patterns, and genetics. The β3-receptor is found primarily in adipocytes but is also present in pulmonary endothelial cells. The proposed β_4-receptor appears to be a conformational state of β1-receptor in myocardial cells. Neither the β3 or β4 receptors have been linked with regulation of ion transport in epithelial cells [7].

The β2-adrenergic receptor is a 1200-bp, single-copy, intronless gene that encodes a 413-amino acid protein with a molecular mass of approximately 46.5 kD. The β2-receptor is a prototypical G protein-coupled receptor (GPCR) with seven-transmembrane domains, an extracellular amino terminus, an intracellular carboxyl terminus, three interconnecting extracellular loops, and three intracellular loops [8–12].

The β2AR is present in high numbers in the bronchial smooth muscle, bronchial epithelium and in endothelial cells. Furthermore, this receptor is present in relatively high numbers in peripheral blood vascular smooth muscle, and to a lesser extent in the heart [13].

The gene that encodes the β2AR is one of the most studied candidate genes in asthma. It is situated on the long arm of chromosome 5 (5q32-q34) [14]. For the gene encoding the β2 adrenergic receptor, several polymorphisms have been described in particular at codons 16 Arginine/Glycine and 27 Glutamine/Glutamic acid which alter the receptor function in vitro [15,16]. The current study will focus on the polymorphism of β2 adrenergic receptor gene (β2AR) at codon 16 that due to preliminary studies which have suggested that β2 adrenergic receptor polymorphism is known to be functionally relevant and also disease-modifying in subjects with asthma. Also, it will assess the relation between certain forms of asthma severity and polymorphism of β2 adrenergic receptor gene.

2. Patients and methods

2.1. Patients

Fifty-nine unrelated children were included in this study. They were divided into two groups:

Group I (study group): This group included 40 asthmatic children. Their age ranged between 2 and 12 years. Thorough history and physical examination were done to exclude other common conditions that mimic asthma. Participants met standard criteria for a diagnosis of asthma. They were classified as having severe (20 child) or mild to moderate asthma (20) according to criteria developed by National Institutes of Health/NHLBI SARP based on American Thoracic Society Consensus Panel report [17].

Group II (control group): This group included 19 healthy children of matched age and sex to the study group.

2.2. Methods

Molecular studies were carried out for 59 cases; using a polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) to detect the polymorphisms of β2AR gene at codon 16.

Venous blood samples were collected into sterile EDTA tubes and stored at −20 °C until used. Genomic DNA was isolated from blood samples by using EZ-10 spin column DNA kit (Biotechnology INC., Canada) to detect the Arg16Gly polymorphism. DNA was analyzed with specific-PCR technique according to the method described by Reihsaus et al. [18].

The nucleotide sequences of the forward and reverse primer used for PCR are: 5-CCTTCTTGCTGGCAGCCCATC-3 and 5-GGAAGTCCAAAACTCGCACCA-3.

The amplification was reformed by including the reaction mix for 35 cycles in a thermocycler. Each cycle consisted of denaturation of DNA at 94 °C for 30 s followed by annealing at 58 °C for 30 s and extension at 72 °C for 30 s with initial delay for 2 min at 94 °C at the beginning of the first cycle and 10 min delay at 72 °C at the end of the last cycle. Amplified products were stored at −20 °C.

The amplified product by PCR was separated on 1.5% agarose at 100 V for an hour to run along with the ladder DNA. The PCR products were visualized on ultraviolet transilluminator, and then photographed by using digital camera (Canon, Power Shot, and 8.0 Mega Pixels). The generated PCR product size using these primers is 640 bp. This PCR product was digested with NcoI at 65 °C (Fermentas, Canada), the Arg16 allele is

distinguished by three bands at 108, 122, and 170 bp; the Gly16 allele is distinguished by two bands at 306 and 334 bp.

2.3. Statistical analysis

The results were analyzed using the Statistical Package of Social Sciences (SPSS) computer software program, version 16.0 (Chicago, IL, USA). Data are presented as numbers and percentages. Association between categorical groups was evaluated using Chi-square (χ^2) test; we also calculated odd ratio (OR) and 95% confidence interval (CI). A *P*-value less than 0.05 was considered statistically significant while, *P* value of <0.001 indicates a high significant result.

For inclusion in the study, an informed written consent was obtained from the children's guardians. The study protocol was approved by the hospital's ethical committee.

3. Results

Results are summarized in Tables 1 and 2. There was a highly statistically significant difference of polymorphisms' distribution of β2AR gene at codon 16 among asthmatic patients and control subjects ($\chi^2 = 11.904$; $P = 0.0026$), also among severe asthmatics and mild/moderate asthmatics ($\chi^2 = 10.108$; $P = 0.0064$). We found also highly statistically significant difference of distribution of heterozygous Arg16Gly among all asthmatics and controls ($\chi^2 = 7.585$; $P = 0.0059$) and also between severe and mild/moderate asthma ($\chi^2 = 8.182$; $P = 0.0042$, Table 1).

3.1. Heterozygous Arg16Gly genotype of β2AR gene

There was a higher frequency of heterozygous Arg16Gly of β2AR in asthmatic children than in normal healthy controls (45% vs. 5.3%, $P < 0.001$), with odd ratio (OR) 14.727 (95% confidence interval (CI): 1.789–121.209). Statistical analysis showed the presence of strong association of heterozygous Arg16Gly of β2AR with severe asthmatics rather than that in control subjects (70% vs. 5.3%, $P < 0.001$), with odd ratio (OR) 42 (95% confidence interval (CI): 4.520–390.297). Also, there was association between heterozygous Arg16Gly with severe asthma when compared to mild/moderate asthmatics (OR, 9.333; 95% CI: 2.179–39.962, $P < 0.05$). Moreover, the highest OR in heterozygous Arg16Gly (42) in comparing severe asthmatic children with controls suggests a dominant mode of action of the heterozygous Arg16Gly in development of asthma severity (Table 2). This indicates also that heterozygous Arg16Gly of β2AR has a high dependent risk factor for the pathogenesis of asthma and has a strong association with severe asthma.

3.2. Homozygous Gly16 genotype of β2AR gene

Homozygous Gly16 has a significant difference between severe and mild to moderate asthma (20% vs. 55%, $\chi^2 = 3.84$, $P = 0.05$). Odd ratio revealed that homozygous Gly plays as a protective factor when comparing severe to mild/moderate asthmatics (OR, 0.205; 95% CI: 0.050–0.834, $P < 0.05$). Moreover, there was a higher frequency of homozygous Gly16 in control subjects than that in asthmatic children (42.1% vs. 37.5%; $P > 0.05$), with odd ratio (OR) 0.825 (95% confidence interval (CI): 0.271–2.511), with no significant deference. But OR revealed that homozygous Gly has low independent risk factor when comparing mild/moderate asthmatics to their controls (OR, 1.681; 95% CI: 0.473–5.967) (Table 2).

3.3. Homozygous Arg16 genotype of β2AR gene

On the other hand, there was higher prevalence of homozygous Arg16 genotype in normal healthy controls than that in asthmatics (52.6% vs. 17.5%; $P < 0.05$), with odd ratio (OR) 0.191 (95% confidence interval (CI): 0.057–0.643), which revealed that homozygous Arg16 may be considered as a recessive protective factor for the pathogenesis of asthma in children (Table 2).

4. Discussion

The frequencies of β2AR genotypes at position 16 in the human population, was 15–20% for homozygous Arginine, 45% for homozygous Glycine and 38% for heterozygous genotype [19]. However there was a marked interethnic difference in the frequency of β2AR polymorphisms among the ethnic healthy group for example the Gly16 allele was more frequent in Caucasian–Americans 54.3% vs. 41.3% in

Table 1 Distribution and comparison of β2AR genotypes at codon 16 in all studied groups.

Genotype	Homozygous Arg		Heterozygous Arg16Gly		Homozygous Gly		Chi-square	
	No.	%	No.	%	No.	%	χ^2	*P* value
Control ($n = 19$)	10	52.6	1	5.3	8	42.1	11.904	0.0026**
All asthmatics ($n = 40$)	7	17.5	18	45	15	37.5		
χ^2	6.133	7.585	0.00284					
P value	0.0133*	0.0059**	0.9575					
Severe asthma ($n = 20$)	2	10	14	70	4	20	10.108	0.0064**
Mild/moderate asthma ($n = 20$)	5	25	4	20	11	55		
χ^2	0.693	8.182	3.840					
P value	0.4053	0.0042**	0.0501*					

P value >0.05 (non significant).
* *P* value <0.05 (significant).
** *P* value <0.001 (highly significant).

Table 2 Frequency of β2AR genotypes at codon 16 in all studied groups.

	Homozygous Arg		Heterozygous Arg16Gly		Homozygous Gly	
Controls (n = 19); n (%)	10	52.6%	1	5.3%	8	42.1%
All asthmatics (n = 40; n (%) (vs. controls)	7	17.5%	18	45%	15	37.5%
Odds ratio	0.191		14.727		0.825	
95% CI	0.057–0.643		1.789–121.209		0.271–2.511	
P value	$<0.05^{*}$		$<0.001^{**}$		>0.05	
Mild/moderate asthma (n = 20; n (%) (vs. controls)	5	25%	4	20%	11	55%
Odds ratio	0.300		4.5		1.681	
95% CI	0.077–1.163		0.455–44.546		0.473–5.967	
P value	>0.05		>0.05		>0.05	
Severe asthma (n = 20; n (%) (vs. controls)	2	10%	14	70%	4	20%
Odds ratio	0.100		42.000		0.344	
95% CI	0.018–0.556		4.520–390.297		0.083–1.429	
P value	$<0.05^{*}$		$<0.001^{**}$		>0.05	
Severe asthma (vs. mild/moderate)						
Odds ratio	0.333		9.333		0.205	
95% CI	0.056–1.971		2.179–39.962		0.050–0.834	
P value	>0.05		$<0.05^{*}$		$<0.05^{*}$	

By Fisher exact test P value >0.05 (non significant).
* By Fisher exact test P value <0.05 (significant).
** By Fisher exact test P value <0.001 (highly significant).

African–Americans [20]. Among normal Egyptians Arg16 allele was more frequent 57% than Gly16 allele 43% [21]. In the current study we found the frequencies of β2AR genotypes at position 16 among Egyptian children, was 52.6% for homozygous Arg16, 5.3% for heterozygous Arg16Gly and 42.1% for homozygous Gly16. Such ethnic geographic differences may explain alterations in the response to β2AR agonists in different ethnic groups.

In this study, there was a significant difference of distribution of β2AR genotypes at codon 16 among all asthmatics and their control subjects, and also among severe and mild/moderate asthmatics, where the heterozygous Arg16Gly genotype was found to have a higher frequency among patients with severe asthma vs. controls and also among patients with severe asthma vs. those with mild/moderate asthma. This was confirmed by Reihsaus et al. and McQuitty et al. [18,22] who demonstrated that the Arginine16/Glycine form of the receptor is associated with nocturnal falls in peak flow rate in subjects with asthma [23] and with increased airway reactivity [24]. Also, Weir et al. [25] demonstrated that Gly16/Glu27 haplotype was more prevalent in severe than in mild asthmatics and these patients that could be at increased risk of fatal or near fatal asthma. Thakkinstian et al. [26] also suggested that the risk of asthma is modified by the allele at position 16, and Wang et al. [27] found that both in utero and childhood exposure to tobacco smoke were associated with an increased risk for wheeze in children, and the risks were greater for children with the Arginine16/Glycine phenotype. The same result was also reported in other ethnic groups as the Anglosaxic countries and the fareast [28,29] as they found it to be increased in asthma patients who were admitted for asthmatic attacks [30] or corticosteroid dependent [18] or have nocturnal asthma [22].

So, in this study we demonstrated that heterozygous Arg16-Gly of β2AR gene has probably dominant mode of action in development of asthma severity. Thus, we suggest that this genotype of β2AR could be a predictor of the pathogenesis of asthma and has strong association with severe asthma. This was in agreement with Litonjua [14] who reported that heterozygous Arg16Gly has associations with other asthma-related phenotypes, such as nocturnal asthma and asthma severity.

Homozygous Gly16 genotype showed a significant difference between severe and mild to moderate asthma. But OR revealed that homozygous Gly16 plays as a protective factor when comparing severe to mild/moderate asthmatics (OR, 0.205; 95% CI: 0.050–0.834, $P < 0.05$). This result was in agreement with Thakkinstian et al. [26] who suggested that homozygous Gly16 has a recessive protective effect for asthmatic children. Also, we found the absence of a significant deference between the asthmatics who had Gly16 homozygous of β2AR and that in control subjects, and odd ratio revealed that homozygous Gly was low independent risk factor when compared mild/moderate asthmatics to their controls (OR, 1.681; 95% CI: 0.473–5.967) (Table 2). This result was in accordance with Reishause et al. [18] who found no correlation between Gly16 homozygosity and hospital admissions, indicating that mutations of β2AR are not a primary cause of asthma and Wier et al. [25] found no increase in frequency of Gly16 in fatal or no fatal asthma. Also, Schachor et al. [31] demonstrated absence of a significant difference between the distribution of Gly16 in asthmatic subjects and their controls, i.e., no significant impact of β2AR polymorphisms on asthma severity in Ashkenazi Jews, non-Ashkenazi Jews or Arabs. Pagaria [32] found that β2AR polymorphisms are not strongly associated with asthma incidence or prevalence. Also, Gao et al. [33] reported that Gly16 homozygous of β2AR was low independent risk factor for the pathogenesis of asthma.

However many literatures have contradictory results. Contopoulos et al. [34] found that Gly16 homozygotes had much higher risk factor for asthma severity. Also, many authors Yin et al. [35] Shigemitsu and Afshar [36] reported that Gly16 polymorphism of β2AR was overrepresented in nocturnal asthmatic patients, and correlated with asthma severity [16].

In the current study, we found that homozygous Arg16 has a protective role in Egyptian children. This was in agreement

with Basu et al. [37] who found that, there was no increase in the risk of hospital admission caused by asthma exacerbations in the children with the Arg/Arg16 genotype. The Arg16 variant did not appear to be associated with general asthma severity because there was no difference in the Arg16 allele frequency across the different treatment steps. The Arg16 variant had no significant effects on measures of pulmonary function. Also, Summerhill et al. [38] reported that the Arg/Arg-16 children with asthma may constitute a significant population that is likely to show better asthma control and they suggested that the β2AR Arg16Arg polymorphism influences either lung growth or the rate of decline of lung function with age.

The cause of the various effect of this polymorphism in various geographic regions still remains to be determined and it is possible that the balance between the various pathogenic factors that determine the severity of asthma differs in various regions [31].

5. Conclusion

Our study demonstrated a significant association of heterozygous Arg16Gly and severe asthma. Even in studies which demonstrated that polymorphisms of β2AR are not important risk factors for development of asthma, meta-analyses on the effects of this polymorphism suggested associations with other asthma-related phenotypes, such as nocturnal asthma and asthma severity [33].

6. Recommendation

Future studies need to fully characterize all of the variations in the β2AR gene and perform comprehensive association studies and the further progress in asthma care will require better understanding of the molecular and genetic basis for the clinical heterogeneity seen in this disorder. Also, the relation between acute and chronic inflammation as well as airway hyper-responsiveness and airway remodeling is still unclear.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank all clinicians helped to complete this study for their great co-operation.

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